

Prevalence of streptogramin resistance in enterococci from animals: identification of *vatD* from animal sources in the USA[☆]

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Received 7 February 2007; accepted 19 March 2007

Abstract

There is considerable debate over the contribution of virginiamycin use in animals to quinupristin/dalfopristin (Q/D) resistance in humans. In this study, the prevalence and mechanisms of streptogramin resistance in enterococci from animals and the environment were investigated. From 2000–2004, enterococci from samples were tested for antimicrobial susceptibility. Q/D-resistant isolates (minimum inhibitory concentration ≥ 4 $\mu\text{g/mL}$) were subjected to polymerase chain reaction (PCR) using primers for streptogramin resistance genes (*ermB*, *msrC*, *vatD* and *vatE*). From the analysis, 1029/6227 (17%) Q/D-resistant non-*Enterococcus faecalis* enterococci were identified. The majority of Q/D-resistant isolates were *Enterococcus hirae* ($n=349$; 34%), *Enterococcus casseliflavus* ($n=271$; 26%) and *Enterococcus faecium* ($n=259$; 25%). Using PCR, 55.5% ($n=571$) were positive for *ermB*, 3% ($n=34$) for *msrC*, 2% ($n=20$) for *vatE* and 0.3% ($n=3$) for *vatD*; 39% ($n=401$) were negative for all four genes. The *vatD*-positive samples comprised two *E. faecium* from chicken and one *E. hirae* from swine. The nucleotide sequence of *vatD* from the three isolates was 100% homologous to published *vatD* sequences. These data indicate that Q/D resistance among enterococci from animals remains low despite the long history of virginiamycin use. To date, this is the first report of *vatD* from enterococci in animals in the USA.

Published by Elsevier B.V. on behalf of International Society of Chemotherapy

Keywords: Streptogramins; *vatD*; Enterococci; Animals; *vatE*

1. Introduction

There is increasing concern over antimicrobial resistance in bacteria of animal and human origin, including the possibility of transfer of resistance genes from animal to human bacteria, in particular those that may be pathogenic [1,2]. The reported increases in resistance are thought to result from antimicrobial use in animals, and the use of antimicrobials for growth promotion in animals is considered to impact human health [3–7]. In part, this is due to the possibility of transferring antimicrobial-resistant normal or commensal microflora

of animals, which may have developed resistance to antimicrobials used in animal production, via the food chain to humans. Subsequent development of cross-resistance to therapeutic antimicrobial agents used in the treatment of human infections is thought to occur when commensal bacteria transfer their resistance genes. Cross-resistance is of particular concern with enterococci, which have been recognised as one of the primary causes of nosocomial infections in humans, as enterococci serve as a reservoir of antimicrobial resistance genes [8]. An example of cross-resistance and treatment concerns is evident in vancomycin resistance and streptogramin resistance in enterococci. Recently, Synercid[®] (quinupristin/dalfopristin (Q/D)), a combination streptogramin A and B, was approved for the treatment of vancomycin-resistant *Enterococcus faecium* [9]. Virginiamycin, an analogue of Synercid, has been used in animal production for over two decades and it is therefore

[☆] The mention of trade names or commercial products in this manuscript is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture.

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possible that Q/D-resistant *E. faecium* have already emerged in the animal population, which could be disseminated among humans and thus impact treatment [9,10].

Whilst resistance to the streptogramins was first discovered in staphylococci, several resistance mechanisms to Q/D have been described in enterococci [10–12]. These include *ermB* and *msrC* mediating resistance to the B component (quinupristin), and *vatD* and *vatE* that confer resistance to the A component (dalfopristin). Although resistance to the A component is the only requirement for resistance to streptogramin A and B combinations, resistance to both A and B components has been reported to confer a higher level of streptogramin resistance [12,13]. The prevalence of streptogramin resistance genes in enterococci varies depending on the geographical location and the source of the isolate. In Europe, *ermB*, *msrC*, *vatD* and *vatE* have all been found in enterococci from various sources, including humans, animals and the environment [10]. In the USA, *ermB*, *msrC* and *vatE* have all been described in enterococci, but to date *vatD* has not been detected [10,14]. Furthermore, because the known mechanisms of streptogramin resistance have not been detected in a number of streptogramin-resistant enterococci, additional resistance mechanisms are believed to exist [14].

As part of the animal arm of the National Antimicrobial Resistance Monitoring System (NARMS)—Enteric Bacteria at the US Department of Agriculture—Agricultural Research Service (USDA-ARS), Athens, GA, enterococci are routinely collected and analysed for antimicrobial susceptibility, resulting in a large collection of enterococci from various non-human sources. This collection of enterococci was characterised to determine the prevalence and mechanisms of streptogramin resistance in non-*Enterococcus faecalis* enterococci in the USA.

2. Materials and methods

2.1. Bacterial strains, isolation and identification

Enterococci used in this study represent non-*E. faecalis* enterococcal isolates collected for the animal arm of NARMS from 2000 to 2004. Enterococci were isolated from poultry carcass rinsates, food items and environmental rinsates or from swine and dairy cattle faecal samples collected on-farm and identified using multiplex polymerase chain reaction (PCR) as described previously [15].

2.2. Antimicrobial susceptibility testing

Minimum inhibitory concentrations (MICs) for enterococci were determined by broth microdilution using the Sensititre semi-automated antimicrobial susceptibility test system (Trek Diagnostic Systems Ltd., Westlake, OH) according to the manufacturer's instructions. A customised 96-well panel of antimicrobials designed for the NARMS

programme was used. Results were interpreted according to Clinical and Laboratory Standards Institute guidelines [16]. The Q/D MIC breakpoint for resistance was defined as $\geq 4 \mu\text{g/mL}$ and the erythromycin resistance breakpoint was defined as $\text{MIC} \geq 8 \mu\text{g/mL}$. *Enterococcus faecalis* ATCC 29212 and ATCC 51299 were used as quality control strains for determination of MICs.

2.3. PCR and DNA sequencing

The template for PCR was prepared by suspending a single bacterial colony in 100 μL of sterile deionised water. Five microlitres of template were used in preliminary amplification reactions using primers for *ermB*, *msrC*, *vatD* and *vatE* as previously described [12,14,17]. Primers for amplification of the entire *vatD* coding sequence were as follows: forward (5'–3') ATT GTA CTA AAA GGA GGT ATT; and reverse (5'–3') CAA GCA ATT TAT TCC TTA TTC. A 695 bp product was amplified using an initial denaturation step of 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 60 °C for 1 min, extension at 72 °C for 30 s and a final extension step at 72 °C for 10 min. Positive controls for PCR were *Streptococcus pyogenes* AC1 (*ermB*) [17], *E. faecium* 825 (this study) (*msrC*), *E. faecium* CVM 3001 (*vatD*) and *E. faecium* CVM 3002 (*vatE*). Isolates CVM 3001 and 3002 were both kindly provided by Dr. Shabbir Simjee. DNA molecular weight marker XVII (500 bp; Roche, Indianapolis, IN) was used as the standard. All PCR products were sequenced at the ARS Regional Sequencing Facility, Southeast Poultry Research Laboratory, Athens, GA. The *vatD* sequences from this study were compared with published *vatD* gene sequences [13,18] using the National Center for Biotechnology Information's Basic Local Alignment Search Tool (BLAST) analysis and aligned using Align Plus (Scientific and Educational Software, Durham, NC).

2.4. Plasmid extraction, pulsed-field gel electrophoresis (PFGE) and Southern analysis

Plasmids were extracted using alkaline lysis as previously described [19]. Restriction enzymes were obtained from Roche (Indianapolis, IN) and used according to the manufacturer's instructions. A supercoiled DNA ladder (Invitrogen, Carlsbad, CA) and DIG-labelled *Hind*III-cleaved lambda DNA (Roche) were used as molecular weight markers. PFGE using *Sma*I-digested DNA was performed as described previously [20]. *Saccharomyces cerevisiae* chromosomes (BioWhittaker, Rockland, ME) were used as molecular standards for PFGE. Cluster analysis was performed with BioNumerics software (Applied Maths, Sint-Martens-Latem, Belgium) using Dice coefficient and the unweighted pair group method with arithmetic mean (UPGMA). Optimisation settings for dendrograms were 2% with a band tolerance of 3.1%. Probes for Southern hybridisation were generated by substituting standard dNTPs with DIG-labelled dNTPs (Roche) in the amplification reaction according to manufac-

Table 1
Distribution of macrolide and streptogramin resistance genes among enterococcal species

| Species | No. positive (%) ^a | | | | |
|--|-------------------------------|-------------|-------------|-------------|------------|
| | <i>vatD</i> | <i>vatE</i> | <i>ermB</i> | <i>msrC</i> | Negative |
| <i>E. faecium</i> (n = 259) | 2 (0.8) | 18 (6.9) | 117 (45.2) | 31 (12.0) | 91 (35.1) |
| <i>E. hirae</i> (n = 349) | 1 (0.3) | 2 (0.6) | 311 (89.1) | 0 (0) | 35 (10.0) |
| <i>E. casseliflavus</i> (n = 271) | 0 (0) | 0 (0) | 46 (17.0) | 0 (0) | 225 (83.0) |
| <i>E. gallinarum</i> (n = 49) | 0 (0) | 0 (0) | 35 (71.4) | 1 (2.0) | 13 (26.5) |
| <i>E. durans</i> (n = 43) | 0 (0) | 0 (0) | 29 (67.4) | 0 (0) | 14 (32.6) |
| <i>E. avium</i> (n = 16) | 0 (0) | 0 (0) | 13 (81.3) | 0 (0) | 3 (18.8) |
| <i>E. solitarius</i> (n = 9) | 0 (0) | 0 (0) | 7 (77.8) | 0 (0) | 2 (22.2) |
| <i>E. saccharolyticus</i> (n = 3) | 0 (0) | 0 (0) | 2 (66.7) | 0 (0) | 1 (33.3) |
| <i>E. asini</i> (n = 1) | 0 (0) | 0 (0) | 1 (100) | 0 (0) | 0 (0) |
| <i>E. cecorum</i> (n = 4) | 0 (0) | 0 (0) | 3 (75.0) | 0 (0) | 1 (25.0) |
| <i>E. mundtii</i> (n = 4) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 4 (100) |
| <i>E. pseudoavium</i> (n = 1) | 0 (0) | 0 (0) | 0 (0) | 0 (0) | 1 (100) |
| <i>Enterococcus</i> spp. ^b (n = 20) | 0 (0) | 0 (0) | 7 (35.0) | 2 (10.0) | 11 (55.0) |
| Total (n = 1029) | 3 (0.3) | 20 (1.9) | 571 (55.5) | 34 (3.3) | 401 (39.0) |

^a Percent positive determined by dividing the number positive for each gene by the total number for each species.

^b Serotype not determined.

turer's instructions. Megabase DNA from PFGE and plasmid extractions were transferred to nylon membranes as previously described and probed with DIG-labelled PCR products for *vatD* [21].

3. Results

3.1. Antimicrobial susceptibility and detection of resistance genes by PCR

A total of 6227 enterococci were collected from swine (n = 2624), poultry (n = 2339), environmental (n = 749), dairy cattle (n = 330) and retail food (n = 185) samples. Of the 6227, 1029 (16.5%) Q/D-resistant, non-*E. faecalis* enterococci were identified: 42% from swine (n = 434), 30% from poultry (n = 311), 23% from environmental (n = 234), 5% from retail food (n = 47) and 0.3% from dairy cattle (n = 3). Per source, the highest resistances overall were from environmental (234/749; 31%), retail food (47/185; 25%) and swine (434/2624; 17%) samples. Only 13% (311/2339) and 1% (3/330) of poultry and dairy cattle samples, respectively, were Q/D resistant.

At least 13 different enterococcal species were resistant to Q/D (Table 1). The majority of isolates were *Enterococcus hirae* (349/1029; 34%) followed by *Enterococcus casseliflavus* (271/1029; 26%) and *E. faecium* (259/1029; 25%) (Table 1). Of the four resistance genes tested, ≥11 of the Q/D-resistant enterococcal species identified were positive for *ermB*, and 55.5% (571/1029) of all resistant isolates contained this gene. Less than 4% of isolates were positive for *msrC* (34/1029; 3.3%), *vatD* (3/1029; 0.3%), or *vatE* (20/1029; 1.9%). All four genes were found among *E. faecium* isolates, including 2/3 (66.7%) of the *vatD*-positive strains and 18/20 (90%) of the *vatE*-positive isolates (Table 1). The remaining *vatD*- and *vatE*-positive isolates were identified in *E. hirae*, which also accounted for the

majority of *ermB*-positive isolates (311/571; 54%). Thirty-nine percent of the isolates (401/1029) did not contain any of the Q/D resistance genes tested in the study. This group was composed primarily of Q/D-resistant *E. casseliflavus* (225/401; 56%) (Table 1).

3.2. Characterisation of *vatD*- and *vatE*-positive isolates

The three *vatD*-positive isolates and 20 *vatE*-positive isolates were chosen for further study. Twenty of the isolates were *E. faecium* and three were *E. hirae* (Tables 1 and 2). Eighteen *E. faecium* and two *E. hirae* isolates originated from chicken carcass rinsate samples; the remaining *E. faecium* and *E. hirae* isolates originated from dairy and swine faecal samples and an environmental sample. The majority of the isolates were isolated in 2003, with only one strain each from 2001, 2002 and 2004 (Table 2). Nine of the isolates contained a single Q/D resistance gene (seven *vatE* and two *vatD*), 12 contained a combination of *vatE* and *ermB*, and one each contained *vatD* and *ermB*, and *vatE*, *ermB* and *msrC*, respectively. With the exception of *E. faecium* 4112, all erythromycin-resistant isolates (14/15) contained *ermB*; eight isolates were susceptible to erythromycin and these isolates did not contain *ermB* or *msrC* (Table 2). MICs for Q/D-resistant isolates ranged from 4 µg/mL to 16 µg/mL.

3.3. Sequence analysis of *vatD*

A 695 bp amplicon was obtained using primers specific for the entire *vatD* gene sequence (data not shown). The *vatD* amplicons from all three *vatD*-positive isolates were sequenced and the 630 bp coding sequences were compared. The sequences were 100% homologous to the nucleotide sequence of *satA* (*vatD*) from *E. faecium* strain BM4145 isolated from a human clinical sample. The nucleotide sequence from the three isolates in this study differed by

Table 2
Antimicrobial resistance profiles of streptogramin-resistant enterococci

| Strain | Species | Source | Year | Genotype | MIC ($\mu\text{g/mL}$) ^a | |
|--------|-------------------|-------------------------|------|---|---------------------------------------|--------------|
| | | | | | Synercid | Erythromycin |
| 825 | <i>E. faecium</i> | Dairy cattle, faecal | 2004 | <i>vatE</i> , <i>ermB</i> , <i>msrC</i> | 8 | >8 |
| 615 | <i>E. faecium</i> | Playground slide | 2001 | <i>vatE</i> , <i>ermB</i> | 8 | >8 |
| 5309 | <i>E. faecium</i> | Chicken carcass rinsate | 2003 | <i>vatE</i> , <i>ermB</i> | 4 | >8 |
| 3015 | <i>E. faecium</i> | Chicken carcass rinsate | 2003 | <i>vatE</i> , <i>ermB</i> | 16 | >8 |
| 8918 | <i>E. faecium</i> | Chicken carcass rinsate | 2003 | <i>vatE</i> | 8 | 1 |
| 1328 | <i>E. faecium</i> | Chicken carcass rinsate | 2003 | <i>vatE</i> , <i>ermB</i> | 8 | >8 |
| 3004 | <i>E. faecium</i> | Chicken carcass rinsate | 2003 | <i>vatE</i> , <i>ermB</i> | 8 | >8 |
| 1324 | <i>E. faecium</i> | Chicken carcass rinsate | 2003 | <i>vatE</i> , <i>ermB</i> | 8 | >8 |
| 1725 | <i>E. hirae</i> | Chicken carcass rinsate | 2003 | <i>vatE</i> | 8 | <0.5 |
| 1925 | <i>E. faecium</i> | Chicken carcass rinsate | 2003 | <i>vatE</i> , <i>ermB</i> | 16 | >8 |
| 4728 | <i>E. faecium</i> | Chicken carcass rinsate | 2003 | <i>vatE</i> , <i>ermB</i> | 16 | >8 |
| 7103 | <i>E. faecium</i> | Chicken carcass rinsate | 2003 | <i>vatE</i> | 8 | 4 |
| 4112 | <i>E. faecium</i> | Chicken carcass rinsate | 2003 | <i>vatE</i> | 16 | >8 |
| 5712 | <i>E. faecium</i> | Chicken carcass rinsate | 2003 | <i>vatE</i> , <i>ermB</i> | 16 | >8 |
| 4309 | <i>E. faecium</i> | Chicken carcass rinsate | 2003 | <i>vatE</i> | 8 | 1 |
| 1013 | <i>E. hirae</i> | Chicken carcass rinsate | 2003 | <i>vatE</i> , <i>ermB</i> | 8 | >8 |
| 9713 | <i>E. faecium</i> | Chicken carcass rinsate | 2003 | <i>vatE</i> , <i>ermB</i> | 16 | >8 |
| 3716 | <i>E. faecium</i> | Chicken carcass rinsate | 2003 | <i>vatE</i> | 8 | <0.5 |
| 9722 | <i>E. faecium</i> | Chicken carcass rinsate | 2003 | <i>vatE</i> | 4 | <0.5 |
| 7527 | <i>E. faecium</i> | Chicken carcass rinsate | 2003 | <i>vatE</i> , <i>ermB</i> | 8 | >8 |
| 9909 | <i>E. hirae</i> | Swine, faecal | 2002 | <i>vatD</i> , <i>ermB</i> | 4 | >8 |
| 5209 | <i>E. faecium</i> | Chicken carcass rinsate | 2003 | <i>vatD</i> | 8 | 2 |
| 6605 | <i>E. faecium</i> | Chicken carcass rinsate | 2003 | <i>vatD</i> | 4 | 2 |

MIC: minimum inhibitory concentration.

^a The Synercid resistance breakpoint was defined as MIC $\geq 4 \mu\text{g/mL}$ and the erythromycin resistance breakpoint was defined as MIC $\geq 8 \mu\text{g/mL}$.

15 nucleotides in the 5' region of the gene when aligned with the published sequence of *vatD* from *E. faecium* strain F9631160-1 isolated from chicken faeces (data not shown). The 15 nucleotide sequence (5'-GGTCCGAATCCTATG-3') was located at bases 4–18 following the ATG start codon.

3.4. Genetic relatedness of *vatD*- and *vatE*-positive isolates

One isolate, *E. hirae* 9909 containing *vatD*, was lost after freezer storage and subsequent experiments with this isolate were not possible. Therefore, 22 isolates containing *vatD* or *vatE* were subjected to PFGE and were analysed based upon species, source, year and genotype to determine the genetic relationship between isolates. Two major clusters with $\geq 75\%$ similarity were formed from the analysis (Fig. 1). Cluster A contained seven isolates including the *vatE* control. Three isolates in this cluster were *vatE/ermB*-positive, whilst the remaining three isolates contained *vatE* only. The majority of isolates containing multiple (≥ 2) streptogramin resistance genes were located in cluster B (Fig. 1). Of the 14 isolates in cluster B, eight contained combinations of *vatE* and *ermB* and one (strain 825) contained *vatE*, *ermB* and *msrC*. The *vatD* control and the two *vatD*-positive *E. faecium* isolates were also in cluster B; these two isolates had indistinguishable PFGE patterns (Fig. 1). *Enterococcus faecium* isolates 5209 and 6605 were both isolated in 2003 in the months of March and June, respectively, from NARMS Region 4 (Oklahoma, Arkansas, Louisiana, Texas and Mississippi).

3.5. Localisation of *vatD*

Using PFGE and Southern analysis, the *vatD* probe hybridised to identical *Sma*I fragments between 170 and 216 kb in *E. faecium* isolates 5209 and 6605 (data not shown). To localise further *vatD* in the isolates, plasmids were extracted from the *vatD*-positive isolates *E. faecium* 5209 and 6605, digested with restriction enzymes and also probed with *vatD*. Plasmid profiles obtained for isolates 5209 and 6605 were identical and differed from the plasmid profile of the *vatD* control, CVM 3001 (Fig. 2(A)). The *vatD* probe hybridised to restriction fragments similar in size in CVM 3001 and isolates 5209 and 6605, including a ca. 10 kb *Bgl*I fragment and a >23 kb *Bam*HI fragment (Fig. 2(B)). The *vatD* probe also hybridised to two *Eco*RI fragments (ca. 9 kb and 23 kb) in isolates 5209 and 6605, but only to a ca. 9 kb *Eco*RI fragment in CVM 3001 (Fig. 2(B)).

4. Discussion

In the USA, a number of studies on streptogramin resistance in enterococci from animals have been reported [14,22–27]. The general conclusion from these reports suggests that streptogramin resistance among enterococci, specifically *E. faecium*, is relatively common and streptogramin resistance can be attributed to the use of virginiamycin in animals [10]. A limiting factor among many of these studies was the small sample sizes, a factor that could

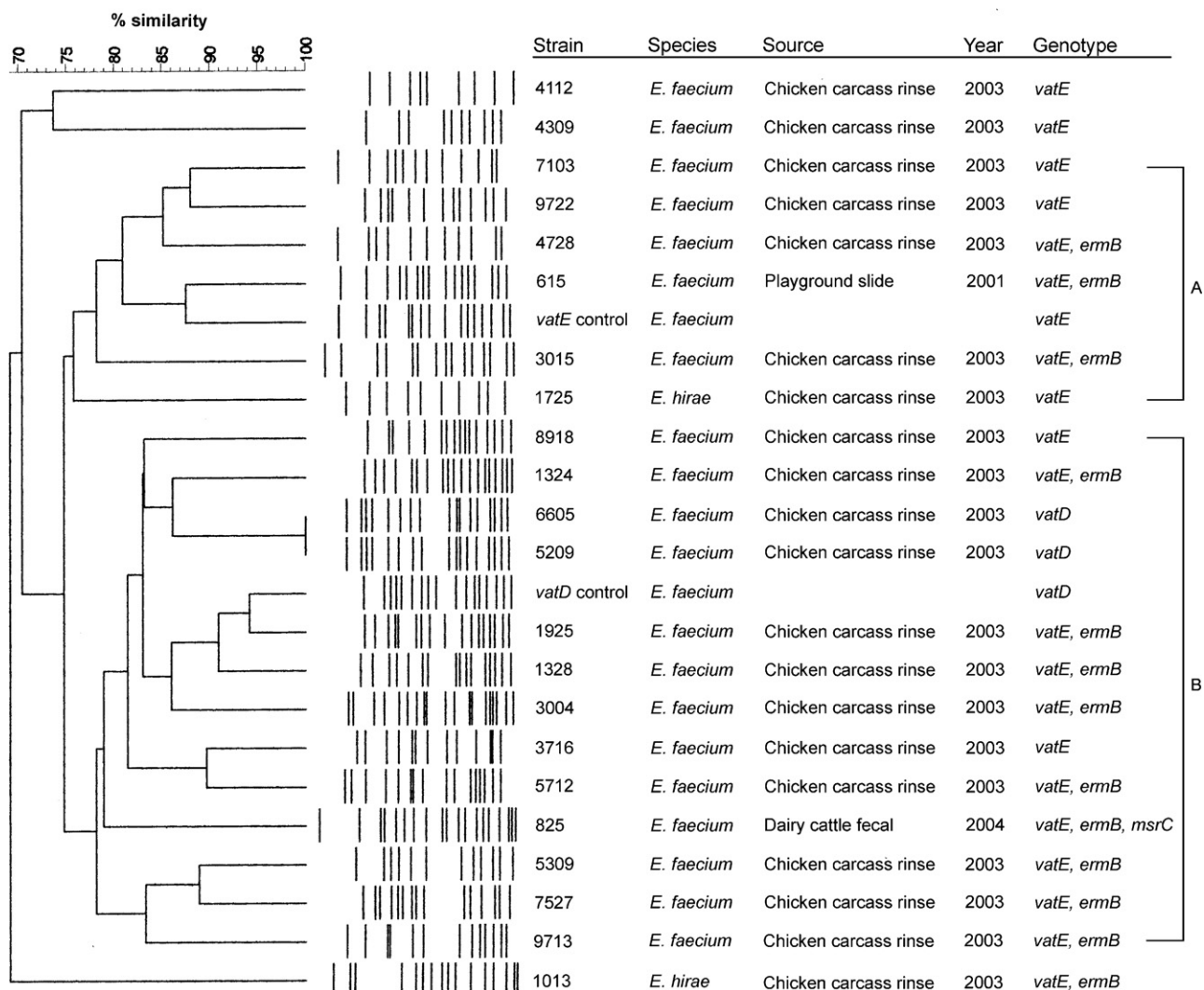


Fig. 1. Pulsed-field gel electrophoresis (PFGE) analysis of streptogramin-resistant enterococci. DNA for PFGE was digested with *Sma*I. Levels of similarity were determined using Dice coefficient and the unweighted pair group method with arithmetic mean (UPGMA).

explain the high rate of streptogramin resistance observed. In the present study, over 6000 non-*E. faecalis* enterococci from various animal and environmental sources from across the USA were tested for susceptibility to Q/D. Of those, 31%, 25%, 17%, 13% and 1% from the environment, retail food, swine, poultry and dairy cattle, respectively, were resistant to Q/D. These percentages were lower than those from other reports where Q/D resistance averaged 8% and 21% for dairy cattle and swine, respectively, and ranged from 58–82% in retail poultry and 33–85% in chickens [10,22,27–29]. The present study also differed from previous reports on Q/D resistance with respect to the enterococcal species studied. Although 25% (259/1029) of the Q/D-resistant isolates were *E. faecium*, only 4% (259/6227) of *E. faecium* from all sources were Q/D resistant. Whilst we did not differentiate between sources of enterococcal species, these data corresponded well with information from another study in which 3% of chicken samples were positive for Q/D-resistant *E. faecium* [28]. Interestingly, the majority of resistant iso-

lates in the present study were *E. hirae*, not *E. faecium*, and few studies on Q/D resistance have included enterococcal species other than *E. faecalis* and *E. faecium* [22,29]. Although *E. faecium* is more likely to be resistant to antimicrobials than *E. faecalis*, and together these enterococcal species account for the majority of nosocomial enterococcal infections, much less research has focused on other enterococcal species [30,31]. The role of other enterococcal species in dissemination and persistence of antimicrobial resistance has not been adequately investigated.

All four Q/D resistance genes tested were detected in *E. faecium* isolates. However, to our knowledge this is the first report of *vatD* in *E. faecium* from animals in the USA. One *E. faecium* containing *vatD* isolated from humans in the USA was recently identified [32]. Overall, the prevalence of *vatD* was very low, as was the prevalence of *vatE* and *msrC*. *vatE* has previously been detected in 26–38% of *E. faecium* from chickens and retail meats and also in *E. faecalis* [10,22,23]. Although *msrC* has been reported as a common Q/D resis-

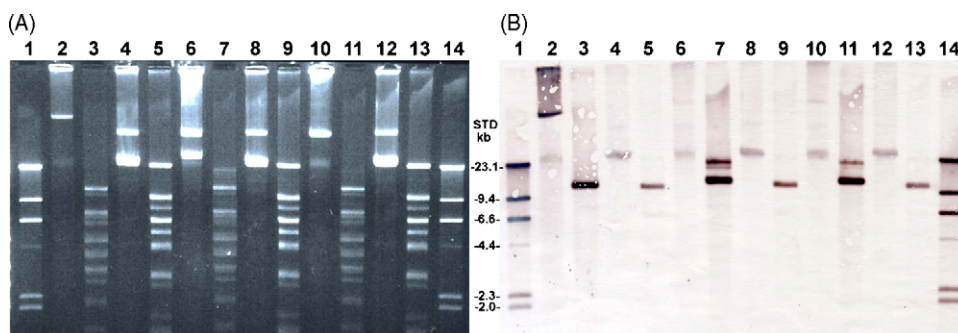


Fig. 2. Southern analysis of plasmids from *Enterococcus faecium* isolates probed with *vatD*. (A) Restriction digests of plasmids from *vatD*-positive strains; (B) restriction digests of plasmids in (A) probed with *vatD*. Lanes 2, 6 and 10, undigested plasmid DNA from CVM 3001 *vatD* control, *E. faecium* 5209 and *E. faecium* 6605; lanes 3, 7 and 11, CVM 3001, *E. faecium* 5209 and *E. faecium* 6605 digested with *Eco*RI; lanes 4, 8 and 12, CVM 3001, *E. faecium* 5209 and *E. faecium* 6605 digested with *Bam*HI; lanes 5, 9 and 13: CVM 3001, *E. faecium* 5209 and *E. faecium* 6605 digested with *Bgl*II; lanes 1 and 14, *Hind*III-cleaved lambda.

tance gene, this was not observed in this study as only 12% of the *E. faecium* contained *msrC* [10,14,33]. Conversely, *ermB* was found in 45% of *E. faecium* and 89% of *E. hirae*, which is consistent with reports of the wide distribution of this gene [10,34]. Thirty-nine percent of the resistant isolates did not contain any of the Q/D resistance genes tested, supporting other findings suggesting that alternative mechanisms of Q/D resistance exist [14,22].

Although the two *vatD*-positive *E. faecium* isolates originated from chicken carcass rinsates and the *E. hirae* isolate containing *vatD* was from a swine faecal sample, the DNA sequences of *vatD* from the three *vatD*-positive isolates were identical to each other. The *vatD* DNA sequences from the three strains were also identical to *satA* (*vatD*) from *E. faecium* strain BM4145 [13]. The *vatD* DNA sequence from all four of these strains differed by 15 nucleotides (bases 4–18 in strain BM4145) in the 5' gene region compared with *vatD* from strain F9631160 [18]. This was very interesting, as strain BM4145 was isolated from a human clinical sample in Europe and strain F9631160 was isolated from a chicken faecal sample from the Danish surveillance programme DANMAP. This suggests that *vatD* in our *E. faecium* and *E. hirae* isolates may be more closely related to *vatD* from humans than from animals.

Two clusters of isolates were detected using PFGE analysis. The majority of isolates from one cluster contained isolates with two or more Q/D resistance genes, whilst the other cluster contained isolates primarily with one resistance gene. In addition to identical *vatD* DNA sequences, PFGE patterns of the *vatD*-positive *E. faecium* isolates were indistinguishable from each other, suggesting that the isolates may be clones. Both strains were isolated in 2003 from chicken carcasses in the months of March and June from the same geographical region as defined by NARMS (NARMS Region 4: Oklahoma, Arkansas, Louisiana, Texas and Mississippi) [35]. Both strains were susceptible to erythromycin and did not contain any other Q/D resistance gene tested in this study. Furthermore, plasmid profiles from the strains were very similar. Southern analysis of the plasmids using a *vatD* probe revealed that the gene was located on plasmids, suggest-

ing that dissemination of *vatD* among enterococci may be possible, although at low frequency.

Results from this study indicate that Q/D resistance among enterococci from animal and environmental sources may not be as prevalent as previously reported [10,36]. It is interesting to note that even with the long history of virginiamycin use in animals, genes mediating resistance to Q/D were not widespread among the isolates. However, detection of the first *vatD* in enterococci from food animals in the USA indicates that Q/D resistance genes are present in these sources. Although conjugation studies were not performed, the presence of *vatD* on plasmids clearly indicates that the gene could disseminate to other enterococci. The origin of *vatD* in enterococci in the USA has not been determined, but the almost simultaneous appearance of *vatD* in humans and animals coupled with the recent introduction of Synercid in human medicine may indicate a different method of dissemination of antimicrobial resistance than that previously hypothesised [5–7]. Finally, additional studies are necessary to identify as yet undetermined mechanisms of Q/D resistance.

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